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—Original Article—

Days in Milk at First AI in Dairy Cows; Its Effect on Subsequent Reproductive Performance and Some Factors Influencing It

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Abstract. The aim of the present study was to show the distribution of cows by days in milk (DIM) at first AI, effect of DIM at first AI on reproductive performance and some factors influencing DIM at first AI. A total of 767 Holstein Frisian cows that calved from January 2004 to December 2007 in 14 dairy herds were used. The first AI conception rate (FAICR) was 34.0%. Seventy-five percent of the cows were first inseminated within 100 days after calving. FAICR increased linearly up to 100 DIM. A one unit (20 days) longer DIM at first AI within the first 100 days postpartum increased the likelihood of a 2.4% FAICR. However, cows first inseminated at an earlier stage of lactation showed better reproductive performance in terms of pregnancy rate and calving to conception interval than cows first inseminated at a later stage. A one day increase in the interval from calving to first AI reduced the likelihood of 0.85 days to become pregnant. Herd or region located in southwestern Japan, tie-stall herd, first AI in spring, higher parity, uterine infection, metabolic diseases and/or mastitis and abnormal resumption of postpartum ovarian cycles contributed to delaying first AI.
Key words: Dairy cows, DIM at first AI, Reproductive performance

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Decreased conception rate at first AI in dairy cows has been reported worldwide [1–4], resulting in an increased number of services per conception, delayed conception and increase of days open. Our recent study also described that approximately 14% of 613 dairy cows in nine dairy herds in Japan had repeat breeding and that the repeat breeders showed extremely poor reproductive performance [5]. Nutritional interactions with reproductive performance in the early postpartum period through high feed consumption required to meet energy requirements have been recognized as reducing the first AI conception rate [6]. Changes in reproductive physiology due to elevated steroid metabolism [7] and metabolic changes in follicular fluid of the dominant follicle in early lactation [8] have been reported to be potential causes of reduction of the first AI conception rate in lactating dairy cows.

Esslemont and Kossaibati [9] have provided a fertility management assessment checklist. To be considered good fertility management, 95% of the cows in the herd after calving must be serviced by keeping the average calving to first service interval at less than 70 days, the overall heat detection must be more than 55% and the pregnancy rate must be 50% or more. The requirements to achieve this target or high reproductive efficiency in a dairy herd are a disease-free transition period, high rates of submission to AI and a high conception rate per service [2]. However, many current dairy herds have difficulty breeding and achieving a high first AI conception rate in an optimum time.

Conception rate has been reported to be higher in the later stages of lactation than in the early stages of lactation [3, 10–12]. Likewise, following an Ovsynch protocol in cows, the stage of lactation had a significant impact on first AI conception rate. Cows inseminated later in lactation had a higher first AI conception rate than cows inseminated earlier [13, 14]. However, it is still not clear whether later first AI is beneficial in terms of pregnancy rate and calving to conception interval, and not all cows are eligible to be inseminated at an appropriate time of lactation. Therefore, it is necessary to describe the pregnancy rate and calving to conception interval of cows at different days in milk (DIM) at first AI and the factors influencing DIM at first AI.

The aim of the present study was to show the distribution of cows by DIM at first AI, show the effect of DIM at first AI on reproductive performance, and analyze some factors influencing DIM at first AI.

Materials and Methods

Herds and animals

The study was conducted using 14 commercial dairy herds in Japan. The herd size ranged from 20 to 60 cows in lactation with an average milk yield ranging from 8,700 to 10,200 kg/cow/lactation. The 14 herds included nine herds in which the incidence of repeat breeding and reproductive performance of the repeat breeders were investigated intensively and reported elsewhere [5]. A

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total of 767 Holstein Friesian cows that calved during the period of January 2004 to December 2007 with 1 to 10 parities and receipt first AI during the study period were involved in the present study. Fifty-two cows that had calved in the same period but had not been inseminated before culling were excluded from the study. All the herds' management records were collected from the farm staffs, by designated veterinarians or routinely by the authors. The voluntary waiting period (VWP) was 40 days in all herds. Some cows that showed very clear signs of estrus were inseminated before the VWP.

Artificial insemination (AI) and pregnancy diagnosis

Cows detected in estrus were inseminated artificially by the farmer using a rectovaginal technique with frozen thawed semen from proven Holstein Friesian sires. Detection of estrus was based on standing estrus in free-stall herds and both on standing estrus and secondary estrus signs in tie-stalls with a paddock. Cows were detected in estrus based on secondary estrus signs in tie-stall herds. If cows were detected in estrus during morning milking, AI was conducted 3 to 6 h later, and if they showed estrus during evening milking, they were inseminated 2 to 4 h later. The cows not observed in estrus until 30 days or more after insemination were examined by palpation or ultrasonography per rectum to determine pregnancy status.

Classification of cows based on DIM at first AI

The cows were classified into seven groups based on DIM at first AI: within 40 days postpartum, between 41 and 60 days, 61 and 80 days, 81 and 100 days, 101 and 120 days, 121 and 160 days and 161 days or more.

Analysis of effect of DIM at first AI on reproductive performance

First AI conception rate, pregnancy rate within 100, 150 and 210 days postpartum, final pregnancy rate within 510 days, calving to conception interval and number of services per conception were used as reproductive parameters as in our previous paper [5].

Analysis of factors affecting DIM at first AI

Herd, region, housing system, season of calving, season of first AI, parity, condition at calving, retention of fetal membrane, uterine infection, metabolic diseases and/or mastitis and resumption of postpartum ovarian cycles within 80 days after calving were included as factors influencing DIM at first AI.

Region was classified into Hokkaido and Yamaguchi Prefectures, which are located in northern and southwestern Japan, respectively. Housing system was divided into three categories: (1) free-stall; (2) tie-stall with paddock in which cows were tied in the stalls during nighttime and were free in the paddock during daytime after morning milking except in summer, during which the cows were tied in the stalls during daytime and were free in the paddock during nighttime, and (3) tie-stall 24 h in which the cows were kept in tie stalls for the whole day. The season of first AI was classified into winter (December to February), spring (March to May), summer (June to August) and fall (September to November). Calving was considered as abnormal if the calf was delivered with assistance of producers and/or veterinarians. Cows were diagnosed as having retention of the fetal membrane if it was not expelled within 24 h after calving. Those cows diagnosed as having endometritis were categorized as having a uterine infection. Cows that suffered from milk fever, displacement of abomasums, clinical ketosis, hypocalcemia, clinical mastitis or their combinations were considered to have metabolic diseases and/or mastitis. Resumption of postpartum ovarian cycles was assessed using progesterone profiles in whole milk [15]. First ovulation within 35 days after calving followed by two or more regular ovarian cycles was considered to be normal resumption. Abnormal resumption included delayed first ovulation beyond 35 days after calving, prolonged luteal phase after first or second ovulation and irregular ovarian cycles within 80 days postpartum.

Statistical analyses

Statistical analysis was carried out using the SPSS 12.0 for Windows statistical package (SPSS, Chicago, IL, USA). Differences in the first AI conception rate, pregnancy rate within 100, 150 and 210 days postpartum and final pregnancy rate within 510 days postpartum among the groups were analyzed using the Chi-square test. The difference in intervals from calving to conception and number of services per conception between the groups were analyzed using one-way ANOVA. Since some cows did not have all the data for each of the factors that were analyzed for the effects on DIM at first AI, the effects of region, herd, housing system, season of calving, season of first AI, parity, condition at calving, retention of fetal membranes, uterine infection, metabolic diseases and/or mastitis and resumption of postpartum ovarian cycles within 80 days after calving were also analyzed using one-way ANOVA. Differences among the means were compared using the Bonferroni post hoc test at the 0.05 probability level. Life table survival analysis was used to show the proportion of cows that did not conceive after calving for the different groups of DIM at first AI. The relationship between DIM at first AI and days to conception was analyzed using a simple linear regression (Microsoft Excel, 2002).

Results

Distribution of cows at first service after calving

Fig. 1 shows the distribution of cows by DIM at first AI and cumulative percentage of cows inseminated. Out of 767 cows, 36 cows (4.7%) were first inseminated within 40 days after calving, before the VWP, ranging from 23 to 40 days, and 540 cows (70.4%) were first inseminated during the period from 41 to 100 days. Approximately a quarter of the cows were first inseminated later than 100 days after calving, ranging from 101 to 287 days. The mean (±SD) interval from calving to first AI was 84.9 ± 39.4, ranging from days 23 to 287.

Reproductive performance in dairy cows based on DIM at first AI

The effect of DIM at first AI on reproductive performance is shown in Table 1. There was a linear increase ($y=2.44x + 27.45$; $R^2=0.93$, $P<0.05$) in first AI conception rate up to 100 days postpartum (Fig. 2). Conception rate at first AI within 40 DIM was

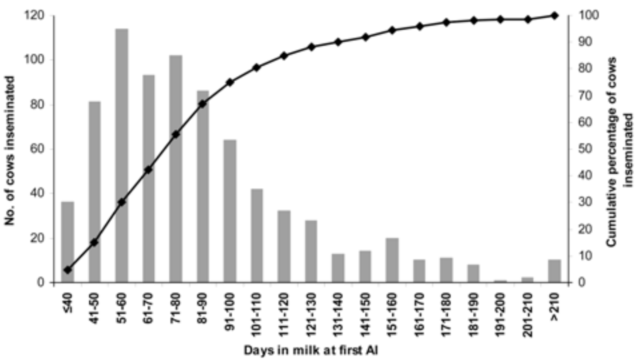

Fig. 1. Distribution of cows by days in milk at first AI and cumulative percentage of cows inseminated.

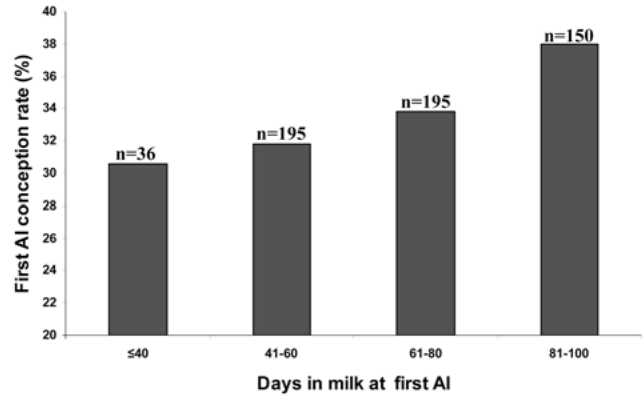

Fig. 2. First AI conception rate in dairy cows for the different days in milk at first AI groups. The first AI conception rate increased linearly up to day 100 (y=2.4385x + 27.453; R^2=0.93, $P<0.05$).

Table 1. Reproductive performance of cows in the different days in milk at first AI groups

	Days in milk at first AI							Total
	≤40	41–60	61–80	81–100	101–120	121–160	>160	
No. of animals	36	195	195	150	74	75	42	767
Calving to first AI interval (days) Mean (± SD)	34.2 ± 3.9	51.5 ± 5.4	70.6 ± 5.7	89.6 ± 5.8	109.2 ± 5.7	138.4 ± 12.5	194.2 ± 33.2	84.9 ± 39.4
First AI conception rate (%)	30.6	31.8	33.8	38.0	31.1	36.0	35.7	34.0
Pregnancy rate ≤100 days (%)	52.8	50.8	43.1	39.3	–	–	–	34.0
Pregnancy rate ≤150 days (%)	69.4^{a}	64.6^{ab}	62.6^{ab}	60.0^{ab}	45.9^{b}	22.7^{c}	–	54.0
Pregnancy rate ≤210 days (%)	83.3^{a}	79.5^{a}	74.9^{a}	72.0^{a}	62.2^{ab}	42.7^{bc}	28.6^{c}	69.0
Final pregnancy rate within 510 days postpartum (%)	83.3^{abc}	90.3^{a}	80.5^{bd}	83.3^{ab}	77.0^{bc}	68.0^{c}	66.7^{cd}	81.4
Calving to conception interval (days) (Mean ± SD)	84.7 ± 50.0^{a}	114.0 ± 73.2^{b}	115.6 ± 58.5^{bc}	129.5 ± 62.2^{c}	154.7 ± 59.4^{d}	172.0 ± 52.3^{d}	236.4 ± 60.0^{e}	129.7 ± 69.8
Services per conception (Mean ± SD)	2.1 ± 1.2^{ac}	2.4 ± 1.3^{bc}	2.1 ± 1.2^{a}	1.9 ± 1.2^{a}	1.9 ± 0.7^{a}	1.8 ± 1.1^{a}	2.1 ± 1.1^{ab}	2.1 ± 1.2
Culling rate (%)	16.7^{a}	9.7^{a}	19.5^{ab}	16.7^{a}	23.0^{ac}	32.0^{bc}	33.3^{bc}	18.6

[a,b,c,d,e] Numbers in rows with different superscripts differ ($P<0.05$).

30.6%, and it increased to 31.8, 33.8 and 38.0% at 41–60, 61–80 and 81–100 DIM at first AI, respectively. A one unit (20 days) longer DIM at first AI within the 100 days postpartum increased the likelihood of a 2.4% first AI conception rate, and the rate was decreased after 100 days postpartum.

There was a tendency for cows that were first inseminated earlier postpartum to have higher pregnancy rates within 100, 150 and 210 days postpartum and to have shorter calving to conception intervals (Table 1). Cows inseminated at 41–60 DIM showed a 7.5 points higher pregnancy rate within 210 days postpartum and a shorter calving to conception interval ($P<0.05$) than those first bred at 81–100 DIM. Cows rebred at 121 DIM or later showed a similar first conception rate to those rebred at 41 to 80 DIM, but the pregnancy rate within 210 DIM was lower and calving to conception interval was longer ($P<0.01$).

Survival analysis showed that a group of cows first inseminated relatively early postpartum required lesser days to become pregnant compared with the other groups of cows rebred during a relatively later period (Fig. 3). There was a positive correlation between DIM at first AI and days to conception ($P<0.01$, Fig. 4). A one day delay in the interval to first AI increased the likelihood of 0.85 days to become pregnant.

Factors influencing DIM at first AI

Among the factors investigated, region, herd, housing system, season of first AI, parity, uterine infection, metabolic diseases and/or clinical mastitis and abnormal resumption of postpartum ovarian cycles were the significant factors influencing DIM at first AI (Table 2).

A larger mean variation in DIM at first AI among the different herds was found in the present study ($P<0.001$), ranging from 63.2 to 123.1 days. Free-stall cows had a shorter DIM at first AI in comparison with tie-stall cows ($P<0.01$). Among the tie-stall cows, a group of cows in 24-h tie-stalls had a lower number of days at first AI than those cows in tie-stalls with a paddock ($P<0.05$). Multiparous cows required significantly longer DIM at first AI compared with primiparous cows ($P<0.01$). The cows with uterine infection or metabolic diseases and/or mastitis or with abnormal resumption

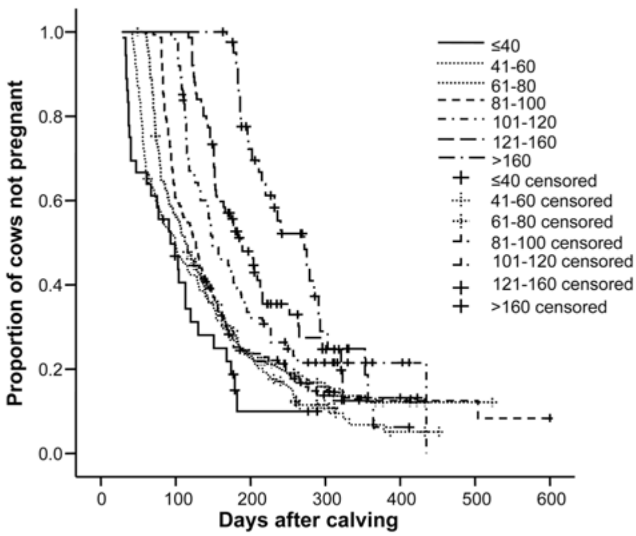

Fig. 3. Kaplan-Meier survival analyses for the proportion of cows not pregnant by days in milk at first AI status. The proportions of cows censored within 40, 41–60, 61–80, 81–100, 101–120, 121–160 and >160 DIM at first AI were 16.7, 9.7, 19.5, 16.7, 23.0, 32.0 and 33.3%, respectively.

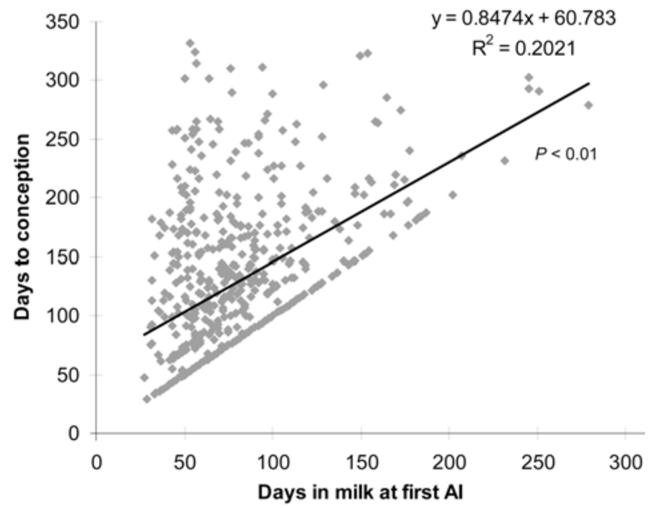

Fig. 4. Relationship between days in milk at first AI and days to conception.

of postpartum ovarian cycles had increased DIM at first AI (P<0.01).

Discussion

The present study was conducted in 14 dairy herds in the northern (Hokkaido) and southwestern (Tottori and Yamaguchi Prefectures) parts of Japan. The results in the present study showed that the rate of cows by DIM at first AI within 100 days in the present study was 75.1%, which was lower than the rates reported by Royal *et al*. [3] in UK dairy herds using two databases, 90.3% (n=1746; periods 1975–1982) and 89.2% (n=621; periods 1995–1998), respectively. However, there was no difference in final number of animals served between the two studies, 93.6 and 95.5%, respectively. In the present study, the mean interval from calving to first AI was 84.9 days, longer than the mean interval from calving to first AI (78.1) reported earlier by Waldmann *et al*. [16], who had investigated 1742 Norwegian dairy cows, and Royal *et al*. (74.0 to 77.6) [3], Darwash *et al*. (71.2) [17] and Esslemont (67.2) [18]. This indicates an extension of DIM at first AI in recent dairy herd management. Increased incidence of delayed resumption of ovarian cycles in today's dairy cows [19] is a possible cause of extended DIM at first AI. Darwash *et al*. [17] found that a one day delay in commencement of luteal activity postpartum caused a delay of 0.24 days in the first postpartum service in 20 commercial herds and the University of Nottingham experimental farm between June 1975 and March 1982. High incidence of abnormal resumption of postpartum ovarian cycles [19], reduced estrous detection efficiency [20], decreased estrus expression [21] and shorter duration of estrus [22] in today's dairy cows may be the reasons for the extension of DIM at first AI in recent dairy cows.

In the present study, the average first AI conception rate for the different DIM groups was 34.0%, ranging from 30.6 to 38.0%, and no statistically significant difference among the groups was found. However, the first AI conception rate in the first group (DIM at first AI ≤40) tended to be lower than those of the other groups. The first AI conception rate in the first group in the present study was only 30.6%, which was lower than those in previous studies, 50.5, 39.7 and 37.0% [3, 12, 18]. Similarly, some previous studies showed that cows first inseminated in an early stage of lactation had a lower first AI conception rate [3, 10–12]. Williamson *et al*. [23], however, reported that early breeding (within 60 days after calving) did not appear to have any adverse effect on subsequent reproductive performance. A tendency for a higher pregnancy rate within 100, 150 and 210 days after calving was shown in cows first inseminated in an earlier stage of lactation than those inseminated later in lactation in the present study. Cows inseminated in an earlier stage of lactation had shorter days open than cows inseminated later in lactation. This is consistent with the study of Dohoo [10]. The linear relationship between DIM at first AI and days to conception in the current study is consistent with the works of Williamson *et al*. [23] and Washburn *et al*. [20].

Causes of delayed first AI after calving in dairy cows that have been reported in several studies include extended voluntary waiting period [24], infection of the reproductive tract [10, 25], season of calving, herd and parity [17], abnormal resumption of ovarian cycles [26, 27] and negative energy balance [4, 7].

In the present study, the average DIM at first AI differed among the different regions, herds, housing systems, seasons of first AI and parities and different statuses of uterine infection, metabolic diseases and/or mastitis and resumption of postpartum ovarian cycles. Herds located in Hokkaido had a shorter DIM at first AI compared with the herds located in Yamaguchi Prefecture. This was probably caused by heat stress due to the different periods of hot humid weather, resulting in a longer DIM at first AI for the herds in Yamaguchi Prefecture. Cows suffering from heat stress

Table 2. Factors influencing days in milk at first AI based on one-way-ANOVA

Factor	Variable	No. of cows	DIM at first AI (Mean ± SEM)	P-value
Herd	Herd A[3]	101	68.5 ± 2.7[a]	<0.001
	Herd B[3]	34	111.2 ± 10.0[bcd]	
	Herd C[3]	29	125.8 ± 10.3[d]	
	Herd D[1]	94	98.7 ± 4.1[b]	
	Herd E[1]	44	68.2 ± 2.8[a]	
	Herd F[3]	63	94.4 ± 4.0[b]	
	Herd G[3]	59	123.1 ± 6.3[cd]	
	Herd H[1]	43	63.2 ± 3.2[a]	
	Herd I[3]	15	102.7 ± 10.9[bc]	
	Herd J[1]	84	75.6 ± 2.5[a]	
	Herd K[3]	87	71.8 ± 3.3[a]	
	Herd L[1]	37	77.2 ± 6.0[a]	
	Herd M[2]	65	65.1 ± 2.6[a]	
	Herd N[3]	12	110.6 ± 9.1[bcd]	
Region	Northern	302	80.1 ± 32.7	<0.001
	Southwestern	400	91.7 ± 44.4	
Housing system	Free-stall	523	76.3 ± 1.4[a]	<0.001
	Tie-stall with paddock	122	108.3 ± 3.9[b]	
	Tie-stall 24 h	122	98.1 ± 4.5[c]	
Season of calving	Winter	214	86.8 ± 2.5	=0.407
	Spring	173	87.7 ± 3.0	
	Summer	190	83.5 ± 3.2	
	Fall	190	81.6 ± 2.7	
Season of first AI	Winter	194	79.9 ± 2.6[a]	=0.004
	Spring	263	91.3 ± 2.8[bc]	
	Summer	117	86.9 ± 3.2[ac]	
	Fall	193	80.0 ± 2.6[a]	
Parity	1	243	77.4 ± 2.3[x]	=0.001
	2,3	337	87.4 ± 2.1[y]	
	4 or greater	182	90.7 ± 3.2[y]	
Condition at calving	Normal	461	85.6 ± 1.8	=0.628
	Abnormal	138	87.4 ± 3.4	
Retention of fetal membrane	No	349	80.0 ± 1.8	=0.076
	Yes	52	89.1 ± 5.3	
Uterine infection	No	439	82.1 ± 1.7	<0.001
	Yes	34	104.2 ± 7.0	
Metabolic diseases and/or mastitis	No	426	81.6 ± 1.8	=0.009
	Yes	47	93.1 ± 4.5	
Resumption of postpartum ovarian cyclicity ≤80 days	Normal	376	78.8 ± 2.0	<0.001
	Abnormal	391	90.8 ± 2.0	

[a,b,c,d] Numbers in a column in the same factor with different superscripts differ (P<0.05). [x,y] Numbers in a column in the same factor with different superscripts differ (P<0.01). [1] Herds located in Hokkaido. [2] Herd located in Tottori Prefecture. [3] Herds located in Yamaguchi Prefecture. The housing system for herds A, D, H, J, K, L, M and N was free-stall. The housing system for herds F and G was tie-stall with paddock. The housing system for herds B, C, E and I was tie-stall 24 h.

had lower oocyte quality [28, 29]; reduced duration and intensity of estrus, altered follicular development and impaired embryonic development [30]; and a higher incidence of abnormal resumption of postpartum ovarian cycles, lower heat detection rate, lower first AI conception rate and lower pregnancy rate [31].

Different housing systems contributed to the initiation of service in postpartum dairy cows. Cows in tie-stall herds had a significantly longer DIM at first AI in comparison with free-stall herds. Increased cow comfort in free-stall herds [32] probably contributed to optimizing DIM at first AI. Herds with tie-stalls were found to have a lower fertility status index, a higher clinical mastitis rate and a significantly higher proportion of herds with ketosis [33].

Season of calving was reported to affect the interval from calving to first service postpartum. Cows calving in spring and winter had shorter intervals to first service than those calving in summer or fall [17]. In the present study, no significant difference in DIM at first AI was found among the different seasons of calving. However, cows calving in summer and fall tended to have shorter intervals to first service than those calving in winter and spring. This was probably due to higher incidence of delayed ovarian activity during the first 50 days after calving in the cows calving in winter [34].

The general goal for postpartum reproductive health in dairy cattle is for the uterus to be completely involuted and free of infection and for the cows to be cyclic by the time they enter the breeding period [35]. Uterine infection is a risk factor for infertility in dairy cows [4]. Cows suffering from uterine infection showed delayed first AI in the current study. This causes infertility at the time when the uterine infection is present and subfertility even after successful resolution of the disease [20]. LeBlanc *et al*. [35] and Gilbert *et al*. [25] showed not only delayed first service of affected cows but also substantial impairment on reproductive performance such as extended days open, reduced overall proportion pregnant, reduced pregnancy to first service and greater number of inseminations per pregnancy.

Abnormal resumption of postpartum ovarian cycles significantly extended the interval from calving to first AI. The reason for the delay in the interval to first ovulation in cows with abnormal resumption can be explained partially by the greater negative energy balance during the early lactation period in modern dairy cows [4, 7], which reduces postpartum LH pulsatility [4] and decreases circulating estradiol [7]. Similarly, persistent luteal activity during both the first and subsequent cycles before insemination was associated with reduced fertility and a higher incidence of late embryo mortality [26, 36]. Multiparous cows were more likely to have a prolonged luteal phase than primiparous cows [34]. Risk factors for abnormal estrous cycles include puerperal problems, negative energy balance and uterine disease [21].

In conclusion, seventy-five percent of the cows were first inseminated within 100 days after calving. First AI conception rate increased linearly up to 100 DIM. Cows first inseminated in an earlier stage of lactation showed better reproductive performance in terms of pregnancy rate and calving to conception interval than cows first inseminated in a later stage of lactation. Herd or region located in southwestern Japan, tie-stall herd, first AI in spring, higher parity, uterine infection, metabolic diseases and/or mastitis and abnormal resumption of postpartum ovarian cycles contributed to delaying first AI.

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